

FIGURE 1: Expression of a 43 kDa protein corresponding to mature Der p1 in fusion with the prepeptide MF-alpha of *Pichia pastoris* (construct pNIV4811) in yeast cells. The culture supernatants from various *Pichia pastoris* clones incubated in the absence or presence of methanol (methanol induction for 1 to 5 days indicated on the x axis) have been analyzed by SDS-PAGE and immunoblot analysis using an anti-Der p1 peptide (117-133) polyclonal antibody.

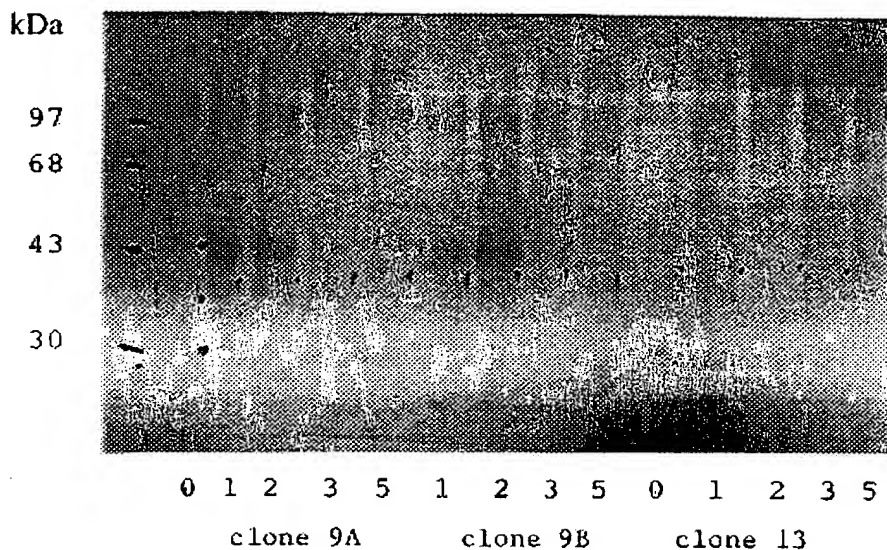


Figure 1

FIGURE 2: Expression of mature Der p1 (30 kDa) in fusion with the prepeptide of *Pichia pastoris* MF-alpha (construct pNIV4817) in yeast cells. The culture supernatants from *Pichia pastoris* cells incubated in the absence (J0) or presence of methanol for 1 day (J1) have been concentrated 50 times and, then, analyzed by SDS-PAGE and immunoblot analysis using an anti-Der p1 peptide (117-133) polyclonal antibody. Arrows indicate the mature Der p1 doublet at about 30 kDa

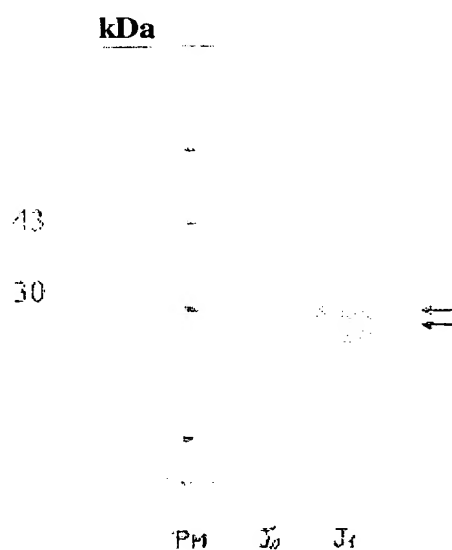


Figure 2

FIGURE 3: Expression of Der p1 in fusion with its propeptide (construct pNIV4812) in CHO-K1 cells. The cell extracts from different clones of CHO-K1 cells transfected with pNIV4812 (lanes 3-8) or transfected with the vector pEE14 alone as negative controls (lane 1 & 2) have been analyzed by SDS-PAGE and immunoblot analysis using an anti-Der p1 peptide (117-133) polyclonal antibody. The arrow indicates the mature Der p1 protein.

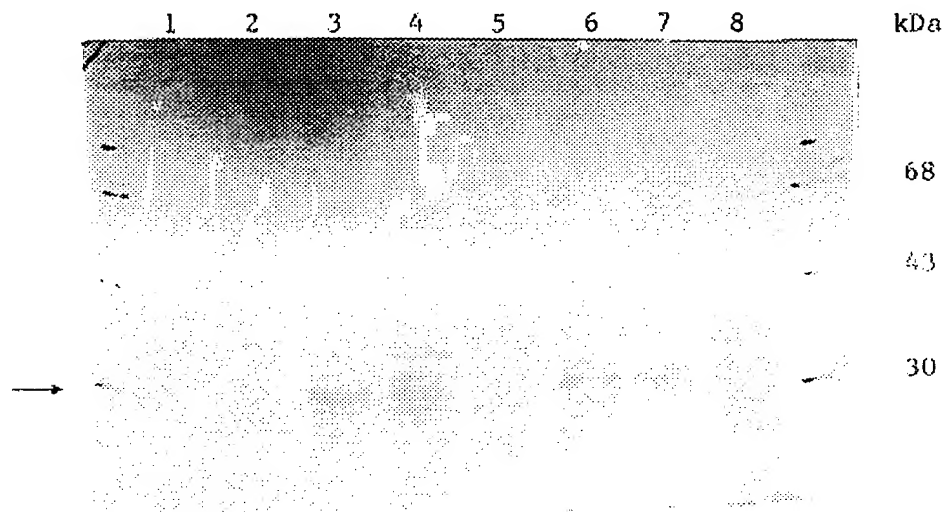


Figure 3

FIGURE 4: Expression of Der p1 in fusion with its propeptide (construct pNIV4840) in drosophila cells S2 (Invitrogen). The cell extracts of different clones of CHO-K1 cells transfected with pNIV4840 (lanes 1 & 4) or transfected with the inducible vector pMT/V5-His alone as negative controls (lanes 2,3,5, & 6) have been analyzed by SDS-PAGE and immunoblot analysis using an anti-Der p1 peptide (117-133) polyclonal antibody. The induction has been carried out for 22 hours (1-3) and 28 hours (4-6).

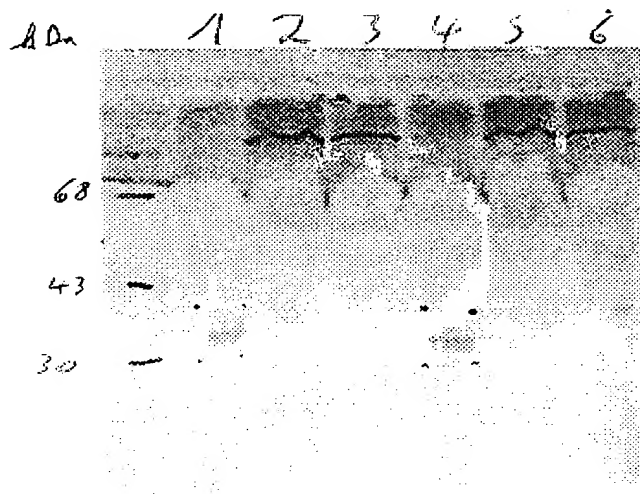


Figure 4

FIGURE 5: Expression of non-cleavable, non-activable Der p1 mutant in fusion with its pro-peptide (construct pNIV4842) in drosophila cells S2 (Invitrogen). The cell supernatants from transiently transfected S2 cells with pNIV4842 (lanes 1-4) or transfected with the inducible vector pMT/V5-His alone as negative control (lanes 5) have been analyzed by SDS-PAGE and immunoblot analysis using an anti-Der p1 peptide (117-133) polyclonal antibody. Lanes 1 to 4 correspond to 1, 4, 5, and 6 days of induction, respectively. Arrows indicate the pro Der p1 doublet at about 36 kDa.

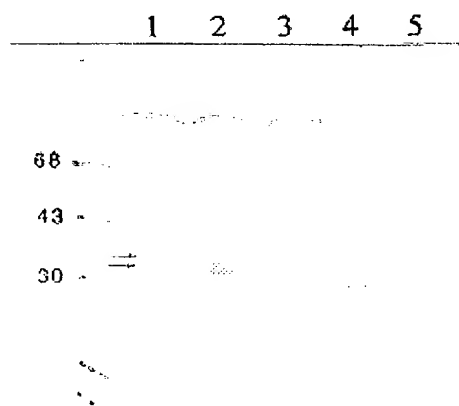


Figure 5

FIGURE 6: Expression of non-active Der p1 mutant in fusion with its propeptide (construct pNIV4843) in drosophila cells S2. The cell supernatants from transiently transfected S2 cells with pNIV4843 (lanes 6-9) or transfected with the inducible vector pMT/V5-His alone as negative control (lanes 5) have been analyzed by SDS-PAGE and immunoblot analysis using an anti-Der p1 peptide (117-133) polyclonal antibody. Lanes 6 to 9 correspond to 1, 4, 5, and 6 days of induction, respectively. Arrows indicate the mature Der p1 doublet at about 36 kDa

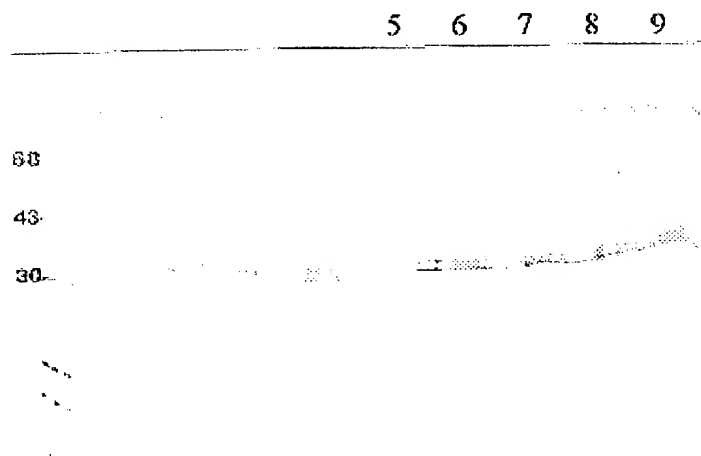
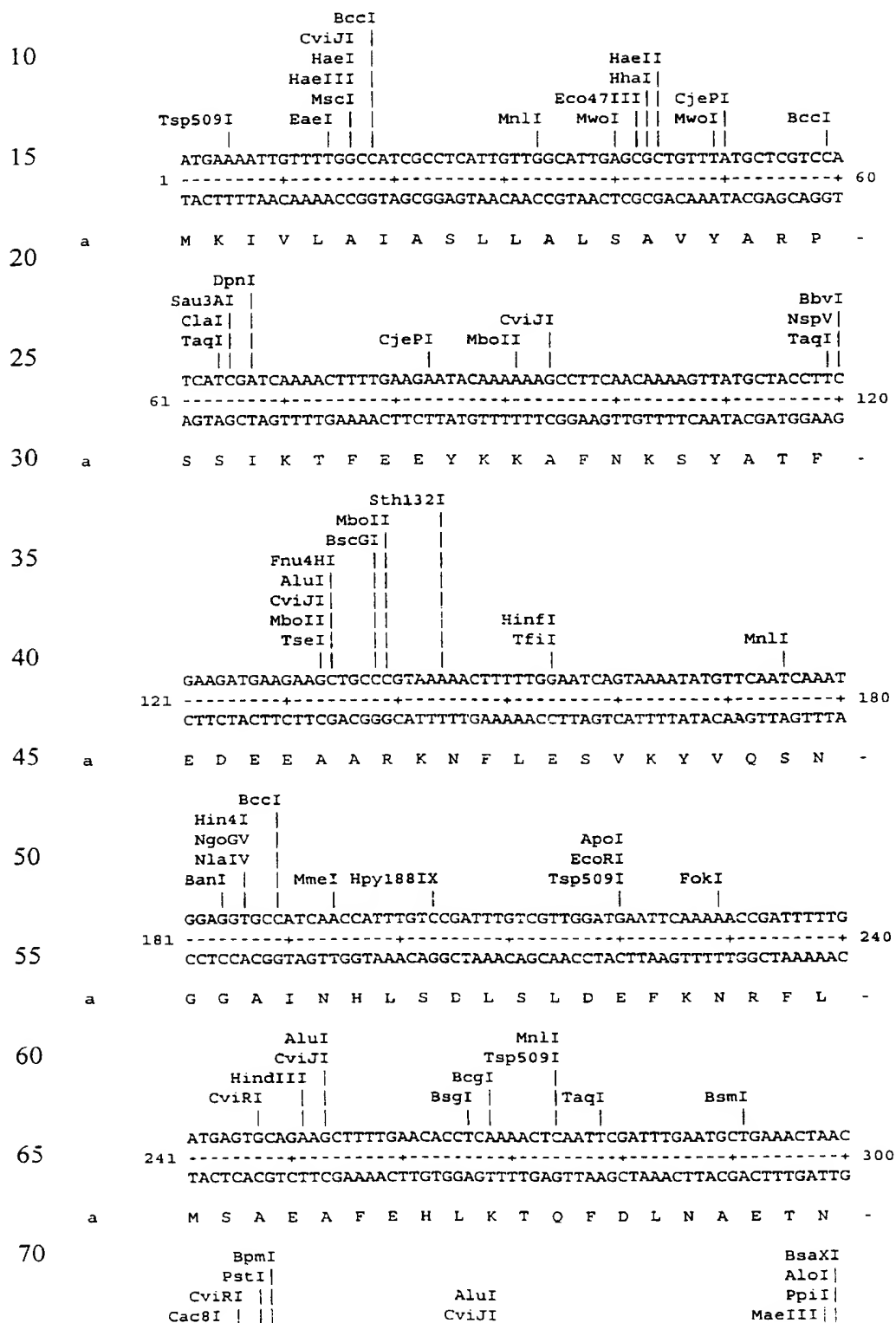


Figure 6

(Linear) MAP of: Derp1.seq check: 7532 from: 1 to: 963

ID DP11695 standard; RNA; INV; 1099 BP.



SfcI | | | MspAII | ClaI | CjePI | TaaI |
 BcgI | | | CjePI | PvuII | TaqI | MwoI | Tsp45I |
 | | | | | | | | | | | | | |
 5 GCCTGCAGTATCAATGGAATGCTCCAGCTGAAATCGATTGCGACAAATGCGAACTGTC 360
 301 -----+-----+-----+-----+-----+-----+-----+-----+
 CGGACGTCATAGTTACCTTTACGAGGTGCACTTTAGCTAAACGCTGTTTACGCTTGACAG
 a A C S I N G N A P A E I D L R Q M R T V -
 10
 CviRI | | | | | | | | | | | | | |
 MnlI | | | | | | | | | | | | | |
 MslI | | | CviJI | DrdII | NlaIII | CviJI | | | | |
 | | | | | | | | | | | | | |
 15 ACTCCCATTCGTATGCAAGGAGGCTGTGGTTTCATGTTGGGCTTCTCTGGTGTGCGCGCA 420
 361 -----+-----+-----+-----+-----+-----+-----+-----+
 TGAGGGTAAGCATACGTTTCTCCGACACCAAGTACAACCCGAAAGAGACCACAACGGCGT
 a T P I R M Q G G C G S C W A F S G V A A -
 20
 DpnI | | | | | | | | | | | | | |
 HinfI | AluI | CviJI | | | | | | | | | | | |
 TfiI | CviJI | MwoI | | | | | | | | | | | |
 | | | | | | | | | | | | | |
 25 ACTGAATCAGCTTATTTGGCTTACCCTAATCAATCATTGGATCTTGCTGAACAAGAATTA 480
 421 -----+-----+-----+-----+-----+-----+-----+-----+
 TGACTTAGTCGAATAAACCGAATGGCATTAGTTAGTAACCTAGAACGACTTGTCTTAAT
 a T E S A Y L A Y R N Q S L D L A E Q E L -
 30
 BsaAI | | | | | | | | | | | | | |
 FokI | | | | | | | | | | | | | |
 PmlI | | | | | | | | | | | | | |
 MaeII | | | | | | | | | | | | | |
 HphI | | | | | | | | | | | | | |
 35 TaqI | | | | | | | | | | | | | |
 | | | | | | | | | | | | | |
 GTCGATTGTGCTTCCCAACACGGTGTGTCATGGTGATACCATCCACGTGGTATTGAATAC 540
 481 -----+-----+-----+-----+-----+-----+-----+-----+
 CAGCTAACACGAAGGTTGTGCCAACAGTACCCTATGGTAAGGTGCACCATAACTTATG
 a V D C A S Q H G C H G D T I P R G I E Y -
 40
 AluI | | | | | | | | | | | | | |
 MaeII | | | | | | | | | | | | | |
 MslI | | | | | | | | | | | | | |
 CjeI | BstXI | | | | | | | | | | | | | |
 | | | | | | | | | | | | | |
 45 ATCCAACATAATGGTGTCTGCTCAAGAAAGCTACTATCGATACGTTGCACGAGAACAATCA 600
 541 -----+-----+-----+-----+-----+-----+-----+-----+
 TAGGTTGTATTACCACAGCAGGTTCTTTTCGATGATAGCTATGCAACGTGCTCTTGTTAGT
 a I Q H N G V V Q E S Y Y R Y V A R E Q S -
 50
 AclI | | | | | | | | | | | | | |
 MaeII | | | | | | | | | | | | | |
 NlaIII | | | | | | | | | | | | | |
 CviRI | | | | | | | | | | | | | |
 | | | | | | | | | | | | | |
 55 TGCCGACGACCAAATGCACAACGTTTCGGTATCTCAAATCTTGGCAAATTTACCCACCA 660
 601 -----+-----+-----+-----+-----+-----+-----+-----+
 ACGGCTGCTGGTTTACGTGTTGCAAAGCCATAGAGTTTGATAACGGTTTAAATGGGTGGT
 a C R R P N A Q R F G I S N Y C Q I Y P P -
 60
 AluI | | | | | | | | | | | | | |
 CviJI | | | | | | | | | | | | | |
 CjeI | | | | | | | | | | | | | |
 HindIII | | | | | | | | | | | | | |
 Hpy178III | | | | | | | | | | | | | |
 ApoI | | | | | | | | | | | | | |
 Tsp509I | | | | | | | | | | | | | |
 | | | | | | | | | | | | | |
 65 HaeII | | | | | | | | | | | | | |
 HhaI | | | | | | | | | | | | | |
 Eco47III | | | | | | | | | | | | | |
 CviJI | BceI | | | | | | | | | | | | | |
 | | | | | | | | | | | | | |
 70 AATGTAACAAAATTCGTGAAGCTTTGGCTCAAACCCACAGCGCTATTGCCGTCATTATT 720
 661 -----+-----+-----+-----+-----+-----+-----+-----+
 TTACATTTGTTTTAAGCACTTCGAAACCGAGTTTGGGTGTCGCGATAACGGCAGTAATAA

09554960 09549600

a N V N K I R E A L A Q T H S A I A V I I -
 5 CviJI
 MaeIII
 BccI
 EaeI
 GdiII
 10 SfaNI BsmI HgaI MslI Thai
 GGCGATCAAGATTTAGACGCATTCCGTCATTATGATGGCCGAACAATCATTCAACGCGAT
 721 -----+----- 780
 CCGTAGTTTCTAAATCTGCGTAAGGCAGTAATACTACCGGCTTGTTAGTAAGTTGCGCTA
 15 a G I K D L D A F R H Y D G R T I I Q R D -
 BstEII MaeIII HincII MaeIII TaaI DraIII
 20 AATGGTTACCAACCAAATATCAGCTGTCAACATTGTTGGTTACAGTAACGCACAAGGT
 781 -----+----- 840
 TTACCAATGGTTGGTTTGTAGTGCAGTGTGAACAACCAATGTCATTGCGTGTCCA
 25 a N G Y Q P N Y H A V N I V G Y S N A Q G -
 CjeI
 TaaI
 BciVI
 30 CjePI AlwI CjeI TaaI
 RsaI HgiEII CjePI
 DpnI MunI HphI
 35 TaqI Sau3AI Tsp509I MaeIII BbvI
 GTCGATTATTGGATCGTACGAAACAGTTGGGATACCAATTGGGGTGATAATGGTTACGGT
 841 -----+----- 900
 CAGCTAATAACCTAGCATGCTTTGTCAACCCTATGGTTAACCCCACTATTACCAATGCCA
 40 a V D Y W I V R N S W D T N W G D N G Y G -
 Bsp24I
 Fnu4HI ClaI CjePI MboII
 45 TseI TaqI CjeI NdeI
 TATTTTGTGCGCAACATCGATTGATGATGATTGAAGAATATCCATATGTTGTCATTCTC
 901 -----+----- 960
 ATAAAACGACGGTTGTAGCTAACTACTACTAAGTCTTATAGGTATACAACAGTAAGAG
 50 a Y F A A N I D L M M I E E Y P Y V V I L -
 TAA
 961 --- 963
 55 ATT

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